EVALUATION OF CHITOSAN AS A COAGULANT FOR DRINKING WATER PRETREATMENT USE WITH CERAMIC FILTER

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ABSTRACT

Xinyu Chen: Evaluation of chitosan as a coagulant for drinking water pretreatment use in ceramic filter

(Under the direction of Mark Sobsey)

Safe drinking water is a major concern in developing countries and household water treatment has been widely promoted as a solution to improving drinking water quality. Ceramic water filters have been identified as a promising technology among the multitude of technologies that are available for household water treatment, however their inability to remove viruses demonstrates the need for more research to augment their performance. A complementary treatment method, in the form of coagulation pretreatment of water, can enhance the removal of viruses. After a brief analysis of three kinds of coagulants, (alum, P&G purifier of water and chitosan salts), three types of chitosan salts, (chitosan hydrochloride, chitosan lactate and chitosan acetate) were identified as technical options worth additional study. Experiments were carried out to evaluate chitosan salts performance and results showed that chitosan lactate and chitosan acetate are promising for further exploration.

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TABLE OF CONTENTS

LIST OF TABLES vii

LIST OF FIGURES viii

LIST OF ABBREVIATIONS ix

CHAPTER 1: INTRODUCTION 1

CHAPTER 2: PROBLEM IDENTIFICATION 2

- **Introduction** 2
- **Global Drinking Water issue** 2
- **Household water treatment** 3
- **Ceramic water filter** 4
- **Coagulation for pretreatment** 6

CHAPTER 3: TECHNICAL OPTIONS DEVELOPMENT 6

- **Introduction** 6
- **Coagulants for Water Treatment** 6
- **Chitosan Background** 9
- **Possible Options** 10
- **Technical Options Identification** 11

CHAPTER 4: RESEARCH DESIGN 13

- **Introduction** 13
- **Materials & Methods** 13

CHAPTER 5: RESULTS & DISCUSSION....18

Results 18

Discussion....22

CHAPTER 6: FUTURE WORK 25

Introduction....25

Next Steps 25

Marketing Plan....26

CHAPTER 7: CONCLUSIONS 30

REFERENCES....31

LIST OF TABLES

Table 1: Description of Treatment Technologies and Methods of Water Purification………………………………………………………………..…..4

Table 2: Chemical Coagulants for Water Treatment and Their Advantages and Disadvantages 8

Table 3: Summary of Performance & Parameters of Concern for Possible Options………………..11

Table 4 Ranges of Influent Turbidity and Initial Log_{10} Concentrations of *E.coli* and MS2 in Three Chitosan Salts Experiments (Filtration Without Chitosan) 18

Table 5: Log_{10} Microbial Reductions and Final Effluent Turbidity of Test Water by Chitosan HCl. Mean (±SD) for 3 Replicate Experiments per Condition..19

Table 6: Log_{10} Microbial Reductions and Final Effluent Turbidity of Test Water by Chitosan Lactate. Mean (±SD) for 3 Replicate Experiments per Condition..20

Table 7: Log_{10} Microbial Reductions and Final Effluent Turbidity of Test Water by Chitosan Acetate. Mean (±SD) for 3 Replicate Experiments per Condition..21

Table 8: Summary of Microbial Reduction by Contribution of Chitosan Candidates Only...24

Table 9: Raw Material Cost of Chitosan Acetate and P&G Purifier of Water...29

LIST OF FIGURES

Figure 1: Chitosan's Structure ……………………………………………..……………………..8

Figure 2: Examples of Modified Chitosan: Chitosan Lactate (a) and
Chitosan Acetate (b) ……………………...…………………..……………………10

Figure 3: Effect of Chitosan HCl on *E.coli* removal……………………..……………………...19

Figure 4: Effect of Chitosan HCl on MS2 removal..……………………………………….....19

Figure 5: Effect of Chitosan Lactate on *E.coli* removal..………………………………………..20

Figure 6: Effect of Chitosan Lactate on MS2 removal..………………………..……………..20

Figure 7: Effect of Chitosan Acetate on *E.coli* removal..………………………………………..22

Figure 8: Effect of Chitosan Acetate on MS2 removal..…………………………………………22

Figure 9: Locations of Ceramic Filters Factories across The World…………………………….27

LIST OF ABREVIATIONS

B2B	Business to Business
B2C	Business to Customer
CDC	Centers for Disease Control and Prevention
CFU	Colony Forming Units
CMWG	Ceramics Manufacturing Working Group
CWF	Ceramic Water Filter
EPA	Environmental Protection Agency
GDWQ	Guidelines for Drinking-water Quality
JMP	Joint Monitoring Programme
HWT	Household Water Treatment
NTU	Nephelometric Turbidity Unit
POU	Point of Use
SODIS	Solar water disinfection
SAL	Single Agar Layer
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth
UNICEF	United Nations International Children's Emergency Fund
WHO	World Health Organization

CHAPTER 1: INTRODUCTION

Ceramic water filters have been identified as a promising technology for household water treatment for use in settings where people rely on surface water that is often fecally contaminated as their primary source of drinking water. However, ceramic water filters are not capable of substantial virus removal due to the small size of viruses without sacrificing filter flow rate. The work described in this Technique Report was done through three briefs submitted over the past year. To identify and define the problem clearly, “Brief 1: Problem Identification Brief”(Chen 2014) suggested that coagulation can be introduced as a pretreatment process. After that, “Brief 2: Solution Identification Brief” (Chen 2015a), evaluated three kinds of coagulants and then compared them based on five criteria. After the evaluation, three types of chitosan salts were identified as technical options worth further exploration. Finally, “Brief 3: Implementation Brief” (Chen 2015b) described the implementation plan for evaluating the performance of chitosan salts. This report synthesizes these three briefs to present the background, analysis and research design required to evaluate whether chitosans are qualified coagulants for drinking water pretreatment as a solution to enhance the effectiveness of ceramic water filters.

CHAPTER 2: PROBLEM IDENTIFICATION

Introduction

Safe drinking water is a major concern in developing countries due to inadequate access to improved sources of drinking water. In order to address shortfalls in access to safe drinking water, household water treatment has been widely promoted as a solution to increasing the quality of drinking water. Several household water treatment technologies have been evaluated based on technical effectiveness and experiences in user households; ceramic water filters have been identified as a promising technology due to their low cost, technical performance, and sustainability in long term use (Sobsey, Stauber & Casanova et al., 2008). While ceramic water filters are effective at removing turbidity and large microorganisms (e.g. bacteria and protozoa), the filters are not capable of substantially removing viruses without sacrificing flow rate below practical levels. Additionally, rapid clogging of filters in regions with highly turbid water can be a negative consequence that deters the use of filters. To address this shortfall, a pretreatment process before filtration can be used in combination with filters to enhance the removal of viruses. Hence, finding suitable coagulants for the drinking water pretreatment becomes a key to solve this problem. Natural coagulants may be of great interest because they are naturally occurring, low-cost products, characterized by their environmentally friendly characteristics. Among natural coagulants, chitosan may be considered as one of the most promising coagulation materials because of its non-toxicity, biocompatibility, and biodegradability.

Global Drinking Water Issue

Safe drinking water is a major concern in developing countries. More than 700 million people around the world lack access to improved sources of drinking water and nearly half of this population lives in sub-Saharan Africa. Each year, there are nearly 1 million diarrheal deaths related to unsafe water and sanitation, and the majority of them are children age under 5 years in developing regions. In 2012, 89% of the world's population, approximately 6.1 billion people, used improved sources of drinking water (WHO, UNICEF, 2014). Improved sources of drinking water are defined as types of technology and levels of services that are more likely to provide safe water than unimproved systems or sources, which include household connections, public standpipes, boreholes, protected dug wells, protected springs, and rainwater harvesting. Other sources, including unprotected wells, unprotected springs, vendor-provided water, bottled water (unless water for other uses is available from an improved source) and tanker truck-provided water are considered unimproved water sources.

According to the JMP report (WHO, UNICEF, 2014), there is a large water access gap between urban and rural areas in the world. While 96% of the urban population uses improved sources of drinking water, only 82% of the rural population has access. The gap is even larger between developed and developing countries. In high-income regions, both urban and rural population have 100% access to an improved source of drinking water, while in low-income regions, 87% of the urban population uses improved sources of drinking water and only 61% of the rural population has access.

Household Water Treatment

To address shortfalls in the provision of safe drinking water in developing countries, household water treatment (HWT) or point of use (POU) water treatment has been extensively promoted as a promising solution. There are a variety of such technologies available for use including physical, chemical and biological treatments to improve the microbial quality of drinking water. Table 1 provides a summary of household water treatment technologies and descriptions of their method of purification.

Table 1: Description of treatment technologies and methods of water purification

Treatment	Purpose
Disinfection. Chlorination with Safe Storage.	Chorine added to drinking water followed by storage in containers designed to reduce the risk of contamination.
Combined Coagulant-Chlorine Disinfection Systems.	Commercial units that combine dry coagulation and chlorine as tablets or sachets.
SODIS (Solar Disinfection)	Transparent polyethylene terephthalate (PET or PETE) bottles filled with aerated source water and left in the sun to disinfect the water by solar UV and increased temperature.
Filtration	Ceramic Filter. Porous ceramic (fired clay) filters to remove microbes from drinking water by size exclusion.
Biosand Filter	A household version of the slow sand filter where potentially pathogenic microorganisms are removed by a biofilm layer, which forms in the top few centimeters of the filter.

Ceramic Water Filter

Ceramic water filters (CWFs) are a low-cost technology that can be locally produced in the countries where they are intended for use using locally sourced clay, soil and fine sized organic materials such as sawdust or rice hulls. The combined material is fired in a kiln burning away the organic material and leaving behind small pores. The pore sizes and surface charge of the ceramic determine the ability of the filter to remove pathogens and other particles from the water.

CWFs are often coated with silver to provide an additional disinfection mechanism.

However, because this silver leaches out of the filters over time, the long-term pathogen removal may be based primarily on the filtration characteristics (Sobsey et al., 2008).

Field studies suggest CWFs are able to overcome many practical obstacles such as water quantity produced, ability to treat a range of water qualities, ease of operation, time to treat and cost per liter by requiring only one-time initial purchase. They produce sufficient water for daily household use with little time and effort. Further more, with the existence of supply chain for necessary replacement, they can achieve large scale adoption and continued, long-term use (Lantagne, Quick & Mintz, 2006; Sobsey et al., 2008; Hunter, 2009). Therefore, CWFs are a promising technology for household treatment.

Previous studies have measured the removal of protozoans, bacteria, and viruses by CWFs. In studies on CWFs without silver the microorganism removal can be attributed to filtration. Brown (2007) found 1.8–2.4 log removal of *Escherichia coli* and 1.3–1.9 log removal of MS2 bacteriophage, respectively. In Van Halem's (2006) study, log removals of 2–5.99, and 1.06–2.31 were achieved for *E. coli*, and MS2, respectively, for filters without sliver. In addition, several previous studies also measured the pore size in CWFs. CWFs from the Potters for Peace factory in Nicaragua showed a pore size range from 0.6 to 3 μm in areas of the filters without cracks (Lantagne, 2001); and 0.02–200 μm with 14 μm the predominant size (Van Halem, 2006). The pore sizes in ceramic disks produced in the lab from flour, grog, and clay were primarily 0.02–15 μm, with a few 100–490 μm (Oyanedel-Craver and Smith, 2008). These results indicate that filtration-based removal of viruses (<0.1 μm) should be poor, particularly because the bulk of the water is likely to flow through the cracks or larger pores. Additionally, the US Environmental Protection Agency requires a POU to be capable of a 6 $\log_{10}$ (99.9999%), 4 $\log_{10}$ (99.99%), and 3 $\log_{10}$ (99.9%) reduction for bacteria, viruses, and parasites, respectively (EPA, 1998). Therefore, it is necessary to improve the performance of CWFs on virus removal for better dealing with microbial threats.

Coagulation as Pretreatment

Adequate virus removal performance for CWFs can be achieved through adding coagulation as a pretreatment process. Coagulation is a commonly applied process in the primary purification of water and wastewater treatment. Coagulation using chemical coagulants consists of combining insoluble particles and/or dissolved organic matter into large aggregates by physical-chemical reactions with an added chemical coagulant, thereby facilitating their removal in subsequent sedimentation, floatation/filtration stages.

In natural water, microorganisms and other small particles are usually negatively charged so they repel each other and stay stable. The idea of coagulation is to de-stabilize the particles, which usually involves the introduction and dispersal by slow mixing of one or several chemical reagents, leading to the formation of micro-floc. Bonding the micro-floc particles together by the addition of a flocculation additive forms larger, denser flocs during slow mixing that are easier to separate. A simple physical separation step such as by plain sedimentation or filtration then eliminates the floc.

CHAPTER 3: TECHNICAL OPTIONS DEVELOPMENT

Introduction

In Chapter 2, adding a coagulation process as drinking water pretreatment use with ceramic filters is proposed. This chapter overviews the commonly used chemical coagulants and compares chitosan (novel application), alum (commonly used coagulants), and P&G Purifier of Water [1](a commercial product), as possible coagulants for household use (Lantagne et al., 2006, Crittenden, Trussell & Hand et al., 2012). After the comparison, three chitosan salts, chitosan hydrochloride, chitosan lactate and chitosan acetate are identified as technical options for further exploring.

Coagulants for Water Treatment

The coagulants frequently used are mineral additives including metal salts (i.e. polyaluminium chloride) and synthetic polymers (i.e. polyacrylamide). Table 2 lists the 4 main types of chemical coagulants that are commonly used for water treatment and their advantages and disadvantages (Sobsey, 2002; Crittenden et al., 2012).

However, lime and soda ash are infeasible for household practice because the pH neutralization is too difficult to perform without specific devices. Also, soluble synthetic organic polymers are usually used as additional aids to enhance the effect of coagulation with inorganic salts instead of primary coagulants. Therefore, only aluminum or iron salts and natural polymers

[1] More information: https://www.csdw.org/csdw/pur-packet-technology.shtml. " A Simple Way to Clean Water The Science Behind the P&G Packet Technology", Procter & Gamble's Campaign for Safe Drinking Water website, accessed Dec 5, 2015.

are potential coagulants for the pretreatment use with ceramic filters. Among natural polymers, biopolymers are particularly promising due to their low-cost and biodegradability and chitosan may be may be considered as one of the most promising coagulation materials (Bratby, 2006).

Table 2: Chemical coagulants for water treatment and their advantages and disadvantages

Coagulant	Household Use	Advantages	Disadvantages
Alum/alum potash & Iron salts	Rare-moderate	Simple technology	Difficult to optimize without training and equipment
Lime ($Ca(OH_2)$), lime+soda ash (Na_2CO_3), caustic soda (NaOH)	Rare-moderate	Simple technology	pH control and neutralization a problem; hazardous chemicals
Soluble synthetic organic polymers	No-rare	Improve coagulation with alum and iron salts	Hard to dose; need training & equipment; hazardous chemicals
Natural polymers (carbohydrates) from seeds, nuts, beans, etc.	Yes (in some developing countries)	Effective, available	Source plant required; training and skill required; may be toxic

Chitosan Background

Chitosan is one of the world's most plentiful and low-cost biopolymers that can be chemically described as a nontoxic, heterogeneous, linear, cationic and biodegradable polysaccharide with high molecular weight (Riva et al., 2011) (Figure 1).

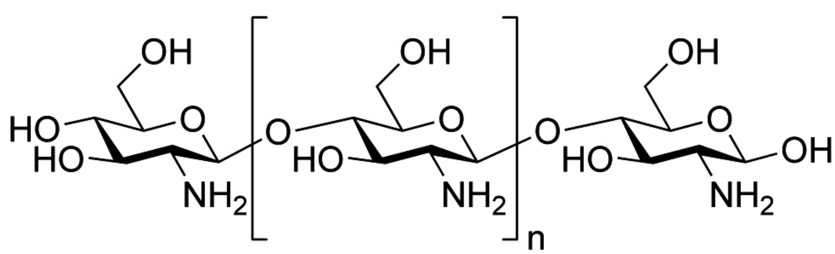


Figure 1: Chitosan's structure[2]

[2] Source: Wikipedia, http://en.wikipedia.org/wiki/Chitosan, accessed Dec 8, 2015.

Chitosan is produced from the alkaline de-acetylation of chitin, which is the second most abundant polysaccharide worldwide. It can be extracted from fungal species or from the exoskeleton of sea creatures such as crayfish, lobster, prawns, crab and shrimp (Muzzarelli, Ilari, & Tarsi et al., 1994). In this process, the acetyl groups of chitin are hydrolyzed and converted to free amine groups (Rinaudo, 2006). When dissolved, the amino groups on the glucosamine units protonate along the chitosan chain, resulting in increasing solubility and positive charge.

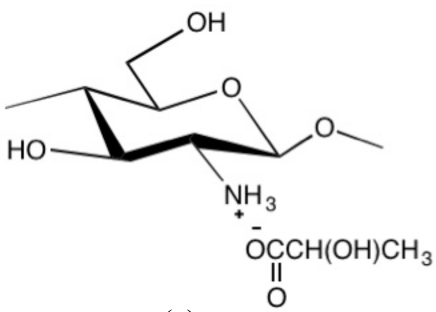
A coagulant with high positive charge density in water at or near neutral pH is more efficient for turbidity and microbial removal than a lower positive charge density or negatively charged coagulant. This is because most natural colloids in water, for example, fine clay, bacteria, silts etc., carry a negative charge over a pH range typical of natural waters, approximately pH 5-9. (Crittenden, Trussell, & Hand, et al., 2012) The combination of the previously mentioned chemical properties contributes to making chitosan a unique adsorbent and coagulant and an ideal candidate for use in water treatment. The specific properties of chitosan such as cationicity, high adsorption capacity, macromolecular structure (Figure 1), abundance and low price (Muzzarelli, Boudrant & Meyer et al., 2012) contribute to make it a unique coagulant and ideal candidate for use in water treatment.

However, the insolubility in water of chitosan polymers means it has to be dissolved in weak acid first before applying, which adds steps to the treatment process and potentially increases cost. Therefore, chemical modification is an effective solution to produce water-soluble chitosans. In chemical modification, reactions occupy functional groups, mainly amino groups, in which aldehydic functions react with the amino groups (Rinaudo, 2006).

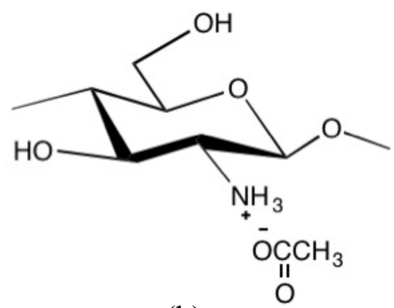
Possible Options

Chitosan salts

Chitosan salt is the simplest form of modified chitosan for higher solubility in water. Modified chitosan dissolves in water over a wide pH range. In order to make a chitosan salt, chitosan polymer is treated with acid so that acid functional groups (e.g. lactate group ($CH_3CH(OH)COO$- or acetate group (CH_3COO-)) are added into chitosan chains (Figure 2), resulting in a chitosan polymer becoming a chitosan salt bearing specific functional groups from the acid used to modify de-acetylated chitosan.



(a)



(b)

Figure 2: Examples of Modified Chitosan: Chitosan Lactate (a) and Chitosan Acetate (b)

Alum

Alum has been used for purification of drinking water and industrial process water at least since the Roman Empire (Faust & Aly, 1998) and is commonly used as coagulant in drinking water treatment in modern facilities. Alum is both a specific chemical compound and a class of chemical compounds. The specific compound is the hydrated potassium aluminum sulfate (potassium alum) with the formula $KAl(SO_4)_2\cdot 12H_2O$. More widely, alums are double sulfate salts, with the general formula $A_2(SO_4).M_2(SO_4)_3\cdot 24H_2O$, where A is a monovalent cation such as potassium or ammonium and M is a trivalent metal ion such as aluminum or chromium(III) (Austin,1984).

P&G Purifier of Water

P&G Purifier of Water is a small sachet of powdered ingredients that make contaminated water potable through coagulation and disinfection. It uses the same approach and ingredients as many municipal water-treatment facilities — the two primary components are ferric sulfate, a widely used coagulant, and calcium hypochlorite, a disinfectant. The powder also contains clay and polymer served as a buffer to provide thorough coagulation and flocculation.

Analysis of Possible Options

Table 3 is a summary of performance and parameters of concern of the possible options. Chitosan salts have a competitive performance on virus reduction but less effective on bacteria reduction when compared to P&G Purifier of Water. All the options are effective in turbidity removal and have a similar optimum pH range. When considering ease of operation and environmental impact, chitosan salts are preferable thanks to their biodegradability and low dose required.

Table 3: Summary of Performance & Parameters of Concern for Possible Options

Performance & Parameters	Chitosan salts	Alum	P&G Purifier of Water
Bacteria $\log_{10}$ Reduction	3.0-4.3[3]	>2[4]	>6[5]
Virus $\log_{10}$ Reduction	2.7-3.8[3]	>2[4]	>4[5]
Residual NTU	0.40-1.14[3]	<1	0.0-3.2[5]
Optimum pH range	4-8[6]	5.5-7.7[7]	5-8.5[8]
Ease of Operation & Environmental impact	Directly combined with CWFs but required periodic cleaning; Biodegradable	Inappropriate to combine with CWFs due to Large dose required; Alternative filters (such as cotton cloth) increase cost and volume of waste	

3 Results of jar tests on Soros' report (2014)
4 Sproul (1974), Leong (1982), Payment and Armon (1989) cited in Sobsey (2002)
5 Results of laboratory test, source: http://www.pghsi.com/pghsi/safewater/pdf/International_PPOW_handout.pdf, accessed Dec 8, 2015
6 Fabris, Chow, & Drikas (2010)
7 *MWH's Water Treatment: Principles and Design, 3rd edition*
8 Amirtharajah, & Mills (1982)

Technical Options Identification

According to Table 3, chitosan salts are quite promising as coagulants for household use in combination with ceramic filters. Therefore, we identify chitosan hydrochloride, chitosan lactate and chitosan acetate as candidate coagulants for use with ceramic water filters as three technical options, based on previous work that demonstrated effective coagulation and flocculation ro reduce bacteria and viruses in conventional drinking water jar tests (Soros, 2014).

CHAPTER 4: RESEARCH DESIGN

Introduction

This chapter presents the research design to evaluate the three technical options, which includes materials and methods for experiments. The results of experiments are also presented in this chapter.

Materials & Methods

Chitosan Salts

Chitosan hydrochloride (HCl), chitosan acetate (CH_3COO^-), and chitosan lactate ($CH_3CH(OH)CO_2^-$) (Soros, 2014) were purchased from Heppe Medical Chitosan GmbH.

Ceramic Water Filters

The ceramic water filters were manufactured locally in Chapel Hill, North Carolina according to the Potters for Peace manufacturing process (CMWG, 2011). The filters were made of clay, sawdust, and water. A lower reservoir consisting of a 5-gallon plastic paint bucket served as a safe water storage unit and a spigot was attached at the bottom of the bucket to access the filtered water. The flow rate of 9 filters was tested by saturating the pores, then filling the filter with water up to the rim, allowing filtrate water to flow by gravity for 1 hr into the lower collection reservoir and measuring the filtrate volume. The average flow rate was measured to be 1.75 L per hour and ranged from 1.4 to 2.3 L per hour for the 9 filters tested. We selected filters with the 6 highest flow rates for the chitosan coagulation and filtration evaluation.

Challenge Waters

Test water consisted of phosphate buffered saline and clay to model turbid, natural water. Phosphate buffered saline (PBS), pH 7.5 was prepared by adding the following to 1L of water: $NaCl$; KCl; Na_2PHO_4, anhydrous; and KH_2PO_4 were added according to EPA Method 1623. All were purchased from Fisher Scientific. pH was adjusted using 1M HCl or NaOH. Kaolinite clay was added to yield turbidity in the range of 10 to 15 NTU.

Overview of Experiment

Each experiment consisted of three filters and one dose of chitosan. The doses chose are: 5 mg/L, 10 mg/L, 20 mg/L and 30 mg/L and the total volume of test water is 3L per filter.

Non-pathogenic surrogates for bacteria and virus removal evaluation were selected based on the WHO household water treatment evaluation list of recommended pathogens and surrogates. Test microbes used were the following: *Escherichia coli* strain K011 (ATCC# 55124) as the model bacterium and male-specific (F+) coliphage MS2 (ATCC# 15597B1) as the model enteric virus. Stocks of microbes were diluted and then dosed into test water at initial concentration of about 1×10^6 to determine at least 6 $\log_{10}$ reductions (99.9999%) by water treatment.

Challenge waters were spiked with kaolinite turbidity and test microorganisms at specified target levels. A total of 9 liters of challenge waters were separately prepared in 3 containers (3L per container) matching 3 filters and samples of the untreated water were taken from the 3 containers respectively (influent water) for microbial analysis. Chitosan powder at specified target concentration was dissolved into the spiked challenge water in each container by agitating the water vigorously for 30 seconds and allowed 30 minutes for coagulation, flocculation and precipitation. After then, the entire volume of coagulated, flocculated and precipitated water was passed through filters by gravity flow and filtrate samples of each filter were collected after 4h filtration (effluent water). Microbes were analyzed in the untreated influent water and the matched filtrate of each filter to determine concentrations and $\log_{10}$ microbe reduction values, which were calculated based on the difference between the $\log_{10}$ influent concentration and effluent concentrations. After finishing every experiment, filters were sterilized by autoclaving on a wet cycle at 121°C for ten minutes. The lower filtrate collection reservoir was sterilized using 70% ethanol and rinsed using deionized water to remove residual ethanol.

Microbiologic Methods

Bacteria detection and enumeration. The bacteria stock for the challenge experiments was prepared by adding a small quantity of *E. coli* K011 from frozen, archived stock to tryptic soy broth (TSB) (Difco) and incubating at 37°C on a shaker set to 100 to 150 rpm overnight (18 to 20 hours). Log phase bacteria were prepared by inoculating 50 mL of TSB and adding 0.5 mL of overnight culture and incubating for 1.5 hours at 37°C, after which the culture was mixed with 40% sterile glycerol in a ratio of 1:1, dispensed in 1mL volumes and stored frozen at -80°C. *E. coli* concentration was 10^9 colony forming units (CFU) per mL. *E. coli* spikes for challenge experiments were 3 thawed tubes of frozen *E. coli* into 3 L of challenge water to give an initial concentration of about 1x 10^6/mL. *E. coli* strain K011 was enumerated by the spread plate method on 100 x15 mm Petri plates of Tryptic Soy Agar (TSA) (Difco) supplemented with 50 μG/ml chloramphenicol) at 12-15 mL/plate according to Standard Methods part 9215 C (Lenore et al., 1998). Water samples of 100μL volume were spread plated, plates were inverted and incubated 18 to 24 hours at 37°C and resulting colonies per plate were counted.

Virus propagation and enumeration. MS2 was propagated in log-phase host *E.coli* F_{amp} (ATCC # 700609) in TSB broth containing 15 µG/mL each of streptomycin and ampicillin by incubating at 37°C on a shaker set to 100 to 150 rpm overnight (18 to 20 hours). MS2 was harvested from infected overnight broth cultures by chloroform extraction with 5% chloroform by volume and centrifuging at 3000 RPM for 30min at 4°C. The recovered supernatant as virus stock was dispensed in 200-300 µL amounts and stored at -80°C. The Single Agar Layer (SAL) assay was used for detection and enumeration of MS2 in water samples according to EPA Method 1602 (APHA, 2001). Another batch of *E.coli* F_{amp} was grown overnight for the purpose of log-phase host preparation which was conducted on experiment days. On the day of water sample assay, autoclaved, molten 0.5X TSA was tempered to 55-65°C. Water sample volumes of 100µL were pipetted into 100 mm x 15 mm petri dishes. Log-phase host was prepared and optical density was measured to verify adequate growth. Molten agar medium was transferred to a to 45°C water bath. When the agar reached temperature, $MgCl_2$, streptomycin and ampicillin were added to achieve concentrations of 0.05M, 15 µG/mL and 15 µG/mL, respectively, and a 4% volume of log phase *E. coli* was added to the molten agar medium. This mixture was distributed in 12-15 mL volumes into petri dishes containing water samples, swirled to evenly mix, and the agar was allowed to solidify. Petri dishes were covered, inverted and incubated at 37°C for 18 to 24 hours, after which MS2 plaques were counted and recorded.

Physical-Chemical Parameters

Turbidity of pre-filtered and post-filtered water was measured using a Hach 2100N Turbidimeter. According to WHO guidelines, turbidity in treated water should not exceed 1 NTU (WHO, 2011a). pH was analyzed by a Denver Instrument Model 215 meter.

Statistical Analysis

E. coli K011 and MS2 $\log_{10}$ concentrations were calculated on the basis of counts from three replicates per dilution in both influent and effluent. Log_{10} reductions of *E.coli* K011 and MS2 were calculated by subtracting effluent $\log_{10}$ concentrations from influent $\log_{10}$ concentrations, respectively. Parametric and nonparametric statistical tests were used to evaluate the difference between microbial reductions when data were normally and non-normally distributed, respectively, as determined by a Shapiro-Wilk normality test (Shapiro & Wilk, 1965). All statistics were interpreted using an a priori significance of α=0.05. All statistical testing was performed in R. One-way ANOVA and Friedman test were used for parametric and nonparametric test, respectively, to evaluate the difference between filtration with no pretreatment versus filtration with chitosan coagulation pretreatment. If there existed significant difference, paired t-tests were used to perform multiple comparisons between pairs of every group of results for the experimental conditions and variables included in the experiment.

CHAPTER 5: RESULTS & DISCUSSION

Results

Table 4 demonstrates the ranges of influent turbidity and initial $\log_{10}$ concentrations of both *E.coli* K011and MS2 in all the experiments. The influent turbidity ranges from 8.7 to 18.7 and the initial $\log_{10}$ concentration of *E. coli* K011 and MS2 ranges from 6.0 to 7.5 and 7.6 to 9.0, respectively, in all experiments.

Table 4 Ranges of Influent Turbidity and Initial $\mathbf{Log_{10}}$ Concentrations of *E.coli* and MS2 in Three Chitosan Salts Experiments (Filtration Without Chitosan)

Chitosan type	Initial *E.coli K011* Log_{10} Concentration	Initial MS2 Log_{10} Concentration	Influent Turbidity (NTU)
Chitosan HCl	6.3-7.0 (6.6-7.0)	7.9-8.7 (8.3-8.4)	9.7-13.1
Chitosan Lactate	6.0-7.5 (6.9-7.0)	8.0-8.4 (8.1- 8.6)	8.7-18.7
Chitosan Acetate	6.2-6.9 (6.2-6.4)	7.6-8.6 (8.9-9.0)	8.9-11.1

Tables 5 -7 summarize microbial reductions and residual turbidity, including the results of statistical analysis of $\log_{10}$ reductions for each concentration of chitosan pretreatment plus filtration compared to $\log_{10}$ reductions for filtration with no pretreatment.

As demonstrated in Table 5 and Figure 3, filtration with no pretreatment resulted in a Log_{10} 3.4 (± 0.27) reduction of *E. coli*, whereas the use of chitosan HCl pretreatment doses ranging from 5 mg/L to 30 mg/L resulted in average $\log_{10}$ reductions ranging from 6.2 to 6.8. Additionally, all doses of chitosan HCl resulted a significant ($P<0.05$) reduction of *E. coli* when compared to no pretreatment, but there were no statistically significant differences between the pairs of different chitosan doses. MS2 $\log_{10}$ reduction with no chitosan pretreatment was only

0.10 (± 0.10), while with chitosan HCl pretreatment at doses of 5 to 30 mg/L MS2 removal was much greater, ranging from 1.9 to 2.4 log_{10} (Table 5 and Figure 4). Also, pretreatment with chitosan HCl resulted significant ($P<0.05$) reductions of MS2 when compared to filtration only.

Table 5: Log_{10} Microbial Reductions and Final Effluent Turbidity of Test Water by Chitosan HCl. Mean (±SD)[9] for 3 Replicate Experiments per Condition

Chitosan HCl Concentration and Filtration Status	*E. coli* K011 Log_{10} Reduction	MS2 Log_{10} Reduction	Effluent Turbidity (NTU)	Pretreatment + Filter vs. Filter Only	
				E.coli K011	MS2
Filtration (F) Only (No Chitosan HCl)	3.4 (± 0.27)	0.1 (± 0.10)	0.2 (± 0.06)	--	--
5 mg/L+ F*	6.2 (±1.03)	1.4 (± 0.30)	0.5 (±0.17)	S**	S
10 mg/L + F	6.4 (± 0.05)	2.4 (± 0.26)	0.3 (± 0.08)	S	S
20 mg/L + F	6.7 (± 0.09)	2.0 (± 0.31)	0.1 (± 0.03)	S	S
30 mg/L + F	6.8 (± 0.03)	1.9 (± 0.29)	0.1 (± 0.04)	S	S

* Different baseline
** S = Statistically significant difference

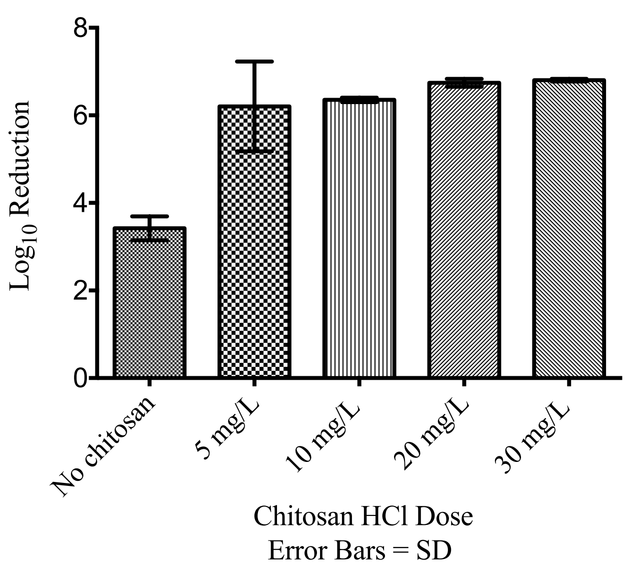


Figure 3: Effect of Chitosan HCl on *E.coli* removal

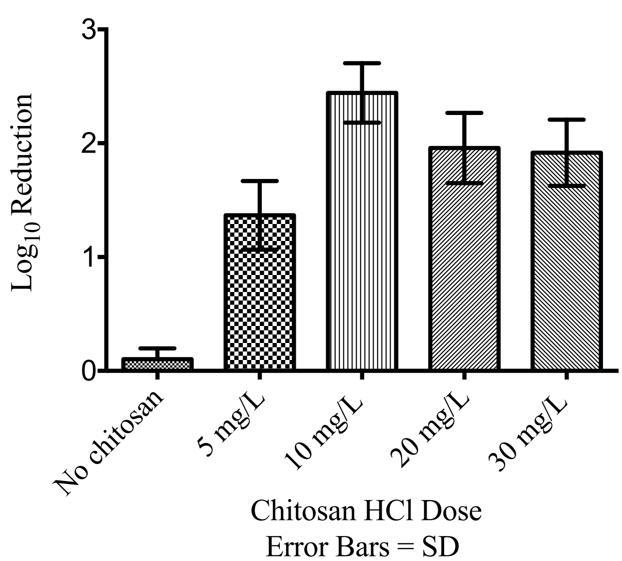


Figure 4: Effect of Chitosan HCl on MS2 removal

[9] The mean (±SD) is reported in all the results as it's inappropriate to apply 95% CI since some of the data fails to meet normal distribution.

However, there was no statistically significant difference in MS2 reductions between different pairs of chitosan doses. From an initial turbidity of 9.7 to 13.1 NTU in the untreated test water, final turbidity levels in all filtrate waters ranged from 0.09 to 0.7 and were below the recommended 1 NTU level of WHO.

Table 6: Log_{10} Microbial Reductions and Final Effluent Turbidity of Test Water by Chitosan Lactate. Mean (±SD) for 3 Replicate Experiments per Condition

Chitosan Lactate Concentration and Filtration Status	*E. coli* K011 Log_{10} Reduction	MS2 Log_{10} Reduction	Effluent Turbidity (NTU)	Pretreatment + Filter vs. Filter Only	
				E.coli K011	MS2
Filtration (F) Only (No Chitosan Lactate)	4.3(± 0.48)	0.2(± 0.16)	0.2(± 0.00)	--	--
5 mg/L + F	6.1(± 0.26)	3.1(± 0.35)	0.2(± 0.00)	S	S
10 mg/L + F	6.3(± 0.28)	3.0(± 0.23)	0.2(± 0.00)	S	S
20 mg/L + F	6.4(± 0.05)	3.2(± 0.25)	0.2(± 0.00)	S	S
30 mg/L + F	7.5(± 0.02)	3.2(± 0.36)	0.2(± 0.06)	S	S

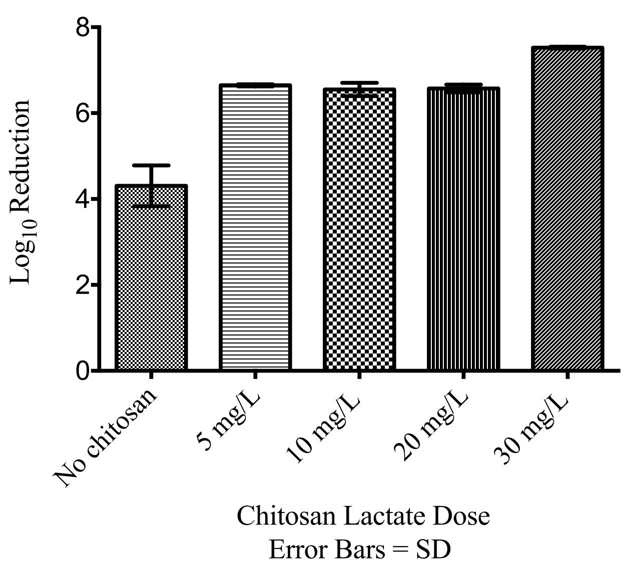


Figure 5: Effect of Chitosan Lactate on *E.coli* removal

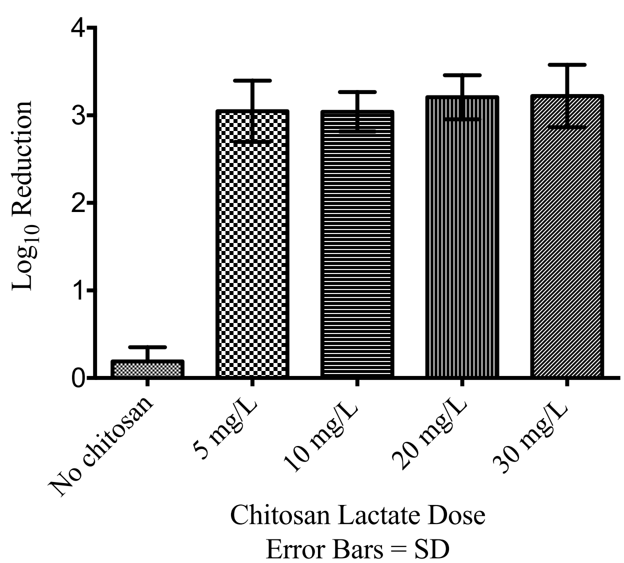


Figure 6: Effect of Chitosan Lactate on MS2 removal

As demonstrated in Table 6 and Figure 5, filtration with no chitosan pretreatment resulted in a log_{10} 4.3 (± 0.48) reduction of *E. coli*. The use of chitosan acetate pretreatment doses ranging from 5 mg/L to 30 mg/L resulted in average log_{10} *E. coli* reductions ranging from log_{10} 6.1 to 7.5, is more than 2 log_{10} greater than filtration alone. Additionally, all doses of chitosan lactate resulted significant ($P<0.05$) reductions of *E. coli* when compared to no chitosan pretreatment. However, there were no statistically significance differences in reductions between different chitosan doses. MS2 log_{10} reduction with no pretreatment was 0.2 (± 0.16) (Table 6 and Figure 6). The log_{10} reductions of MS2 with chitosan lactate pretreatment at doses of 5 to 30 mg/L followed by ceramic filtration ranged from 3.0 to 3.3 log_{10}, which is about a 3 log_{10} increase in MS2 reduction than by filtration alone. Also, all doses of chitosan lactate resulted a significant ($P<0.05$) reduction of MS2 when compared to filtration only, but no statistical significance between different doses. From an initial turbidity of 8.7 to 18.7 NTU in untreated test water, the average final turbidity levels were reduced to 0.2 NTU, well below the WHO recommended 1 NTU.

Table 7: Log_{10} Microbial Reductions and Final Effluent Turbidity of Test Water by Chitosan Acetate. Mean (±SD) for 3 Replicate Experiments per Condition

Chitosan Acetate Concentration and Filtration Status	*E. coli* K011 Log_{10} Reduction	MS2 Log_{10} Reduction	Effluent Turbidity (NTU)	Pretreatment + Filter vs. Filter Only	
				E.coli K011	MS2
Filtration (F) Only (No Chitosan Acetate)	2.4(± 0.62)	0.4(± 0.40)	0.2(± 0.00)	--	--
5 mg/L + F	5.4(± 0.82)	2.8(± 0.10)	0.2(± 0.00)	S	S
10 mg/L + F	4.9(± 1.12)	3.3(± 0.21)	0.2(± 0.00)	NS* (P>0.05)	S
20 mg/L + F	4.7(± 1.56)	3.5(± 0.52)	0.2(± 0.10)	NS (P>0.05)	S
30 mg/L + F	5.4(± 1.31)	4.5(± 1.04)	0.1(± 0.06)	S	S

* NS = No statistically significant difference

As demonstrated in Table 7 and Figure 7, filtration with no chitosan acetate pretreatment resulted in a mean $\log_{10}$ 2.4 (± 0.62) *E. coli* reduction. The use of chitosan acetate pretreatment at doses ranging from 5 to 30 mg/L resulted in average $\log_{10}$ *E. coli* reductions ranging from 4.7 to 5.4, which are more than 2 $\log_{10}$ greater than filtration alone. Only two doses of chitosan acetate (5 and 30 mg/L) resulted significant ($P<0.05$) reductions of *E. coli* when compared to no use of coagulant. However, there were no statistically significant differences in *E coli* reductions between different chitosan doses. MS2 $\log_{10}$ reduction by filtration with no pretreatment was 0.4 (± 0.40) (Table 7 and Figure 8), which is somewhat higher than the $\log_{10}$ reductions reported for experiments with chitosan HCl (0.1 $\log_{10}$) and chitosan acetate (0.2 $\log_{10}$). However, with chitosan acetate pretreatment at doses from 5 to 30 mg/L followed by filtration, MS2 reduction increased greatly to between 2.8 to 4.5 $\log_{10}$. All doses of chitosan acetate resulted in significant reductions of MS2 and there was a statistically significant difference in MS2 reduction between the dose of 5 mg/L and 30 mg/L. From an initial turbidity of 8.9 to 11.1 NTU in test water, final turbidity levels in filtered effluent waters were 0.13 to 0.2 NTU, well below the recommended 1 NTU levels of WHO.

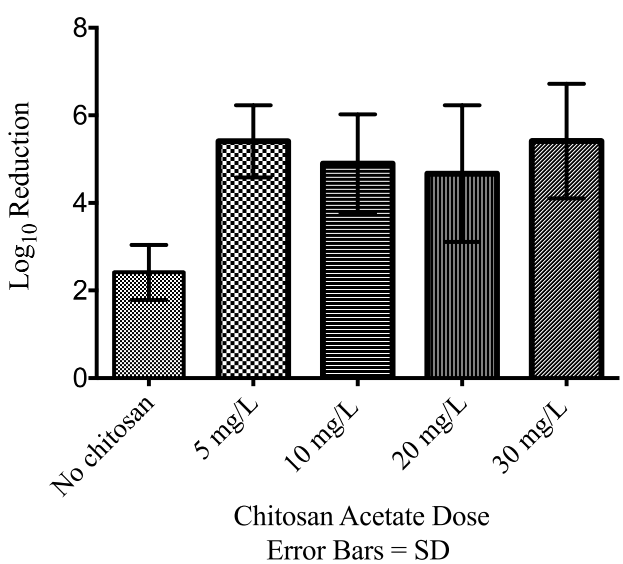


Figure 7: Effect of Chitosan Acetate on *E.coli* removal

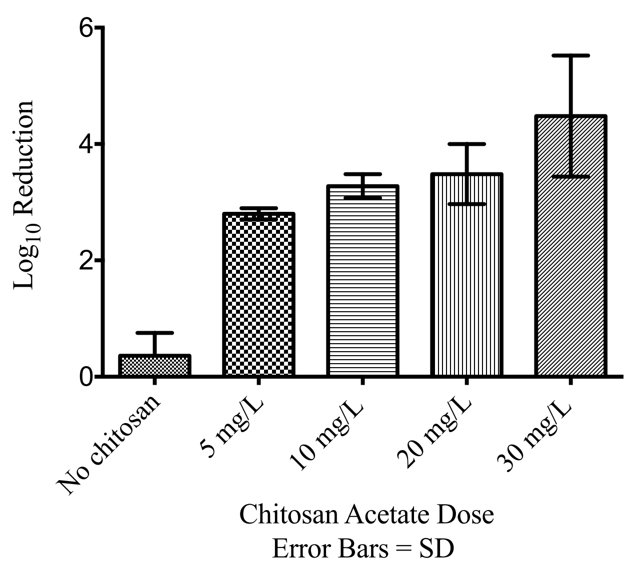


Figure 8: Effect of Chitosan Acetate on MS2 removal

Discussion

In 2011, WHO developed performance targets for the evaluation of household water treatment devices based on health risk targets that are linked to $\log_{10}$ microbial reductions from water. The recommended performance levels consist of a 3-tiered approach for the reduction of bacteria, viruses, and protozoa (WHO, 2011b). The top tier is designated highly protective with bacteria, virus and protozoan parasite $\log_{10}$ reductions of ≥ 4, ≥ 5, and ≥ 4, respectively. Second tier is designated protective and specifies $\log_{10}$ bacteria, virus and protozoan parasite reductions of ≥ 2, ≥ 3, and ≥ 2, respectively. The lowest tier, designated minimally protective, specifies achieving the "protective" $\log_{10}$ reduction levels for two of the three classes of microorganisms as well as providing evidence of health protection, typically from diarrheal disease, by scientifically credible field epidemiological studies.

All three modified chitosans evaluated, chitosan HCl, chitosan lactate and chitosan acetate, achieved extensive reductions of bacteria and viruses. Filtration with chitosan pretreatment consistently produced filtrate water with <0.7 NTU turbidity, below the 1 NTU maximum turbidity limit recommended by the WHO GDWQ. However, filtration alone produced low filtrate water turbidity levels of about 0.2 NTU and therefore, pretreatment with any of the chitosan salts did not further improve turbidity reductions of the filtered water. Pretreatment with any chitosan type plus ceramic filtration achieved the over 4 $\log_{10}$ "highly" protective level of reduction for bacteria set in the WHO HWT performance scheme. Both chitosan acetate and chitosan lactate achieved the 3 $\log_{10}$ reduction "protective" level of performance for viruses of the WHO HWT scheme.

Table 8 summarizes the contribution of chitosan candidates regarding microbial reduction by subtracting the $\log_{10}$ reduction effect of the ceramic filter only. The greatest $\log_{10}$ reduction

Table 8: Summary of Microbial Reduction by Contribution of Chitosan Salts Only.

	E.coli K011 Log_{10} Reduction			MS2 Log_{10} Reduction		
Dose	Chitosan HCl	Chitosan Lactate	Chitosan Acetate	Chitosan HCl	Chitosan Lactate	Chitosan Acetate
5 mg/L	2.3(± 1.30)	1.8(± 0.28)	3.0(± 0.42)	1.3(± 0.30)	2.9(± 0.26)	2.4(± 0.30)
10 mg/L	2.9(± 0.28)	2.0(± 0.74)	2.5(± 0.58)	2.3(± 0.17)	2.9(± 0.09)	2.9(± 0.60)
20 mg/L	3.3(± 0.33)	2.1(± 0.44)	2.3(± 0.96)	1.9(± 0.27)	3.0(± 0.41)	3.1(± 0.91)
30 mg/L	3.4(± 0.27)	3.2(± 0.47)	3.0(± 0.78)	1.8(± 0.36)	3.0(± 0.51)	4.1(± 1.38)

improvement of *E. coli* of 3.4 (±0.27) was achieved with a dose of 30 mg/L of chitosan HCl and the greatest log_{10} reduction improvement of MS2 coliphage of 4.1(±1.38) was achieved with a dose of 30 mg/L of chitosan acetate. However, there were no significant differences between different doses of any chitosan salt with regard to both *E.coli* and MS2 microbial reduction improvements. Hence, a further paired t-test was performed to compare any two chitosan types regardless of the dose. Results showed that there was no significant difference between any two chitosan types on *E.coli* removal, whereas with MS2 removal, both chitosan lactate and chitosan acetate had significant differences from chitosan HCl but no significant difference between each other. Our results demonstrate that, at varying doses of the three modified chitosans evaluated in this analysis, two types of modified chitosans, the acetate and lactate salts, provide the greatest capacity for significant microbial reductions from water by coagulation and flocculation prior to ceramic filtration, especially of viruses, and are worthy of further exploration.

CHAPTER 6: FUTURE WORK

Introduction

In chapter 5, coagulation and flocculation with chitosan salts gave improved results for *E.coli* and MS2 removal by ceramic filtration. However, further research is required before chitosan salts become ready for use at the household level. This chapter proposes next steps for laboratory experiments and field study. In addition, a brief marketing plan was developed to describe how chitosan salts can reach the end-users for "real world" application, which includes the blueprints of production, distribution, education, sales etc.

Next Steps

Currently, this research investigated the effect of chitosan salts on *E.coli* and MS2, which are recommended bacterial and viral indicators, respectively, that are commonly used for household water treatment performance evaluation. However, these indicators only address two classes of microorganisms thereby neglecting protozoa surrogates. In addition, the test water used here was buffered water with clay to model turbidity and was created using a specific clay, kaolinite. While these previous tests allowed for screening chitosans to evaluate their performance, it is necessary to evaluate chitosan salts in natural water and with a range of water quality characteristics by varying total organic carbon (TOC), turbidity, total dissolved solids (TDS), and pH.

Because chitosan lactate and chitosan acetate demonstrated the best performance with regard to microbial removal based on this research, next steps should involve further dose optimization experiments under natural water conditions and with a range of test water quality conditions. Future experiments may involve lake water in combination with 1% municipal sewage, which provides organic matters and turbidity and simulates highly contaminated natural water. Upon completion of dose optimization studies, field studies will follow and be performed in households where ceramic filters are commonly used. A selection of pilot sites is required to assess success of integrating chitosan salts into household water treatment.

Furthermore, further physical-chemical characterization of the properties and performance of the three chitosan salts is required to better understand the mechanisms of microbial reduction. Therefore, floc size, density and electro-potential characteristics require further analysis to be performed and interpreted to characterize these floc properties of the three chitosan salts, which will inform the relationship between type of chitosan and performance outcomes.

Marketing Plan

Target Market and Customers

Because chitosan is proposed to be used in combination of ceramic water filters, the potential markets are all the regions where ceramic filters are widely used. Although not the focus of this assessment, more investigations are required to identify the target opportunities for augmenting the performance of household level filtration technologies other than ceramic filters, such as granular medium filters. Once the target markets are determined, then the next step is to identify the target customers. Assuming a new company is established to sell chitosan products, then there can be two possible business models:

Business to Business (B2B) ---- Filter manufacturers as target customers

Business to Customer (B2C) ----- Filter end-users as target customers

For the scenario where the filter manufacturers are the target customers, Potters for Peace (PfP), the leading non-profit organization that has established ceramic water filter factories worldwide, would be one of the largest customers. Figure 9 demonstrates the locations of ceramic water filters factories of PfP across the world. The factory locations are in Latin America, Africa and Southeast Asia.

Figure 9: Locations of Ceramic Filters Factories across the World

<u>Production & Distribution</u>

Currently, most suppliers of chitosan are in China's coastal areas and in various parts of the United States. A market search should be conducted to contact and identify manufacturers who are interested in this new application. However, from the standpoint of reducing cost, Latin America and Africa may need local suppliers of chitosan, considering the relatively high transport cost for importing chitosan. Because documentation of chitosan manufacturing processes already exists, the technique will not be a barrier and whether conditions are favorable for setting up a local factory depends on several factors as follows:

- Availability of raw material: the region should have a large consumption of crustacean products so it is cost-effective and easy to get the exoskeleton as waste.
- Market size: the potential demand of chitosan product should be large enough to support the operation of a local factory.
- Other aspects: the environmental burden of producing chitosan products, the transportation convenience to dispatch products, the land use permission and power supply for a factory are all factors of concern.

Regardless of where to localize production and take PfP as our target customer for a B2B example, our company needs to consider bridging the chitosan factories and the filter factories of PfP. PfP would take charge of the sales to the end-users through their distribution chains and our company would provide technical support on education and promotion on product use.

For end-users of ceramic filters as the target customer, our company would be directly responsible for selling products to these customers. For a newly established company, it is likely to be preferable to join an existing filter manufacturer/supplier like PfP and use its network for distribution. Otherwise, a new chitosan company may consider approaching other distribution channels such as hardware stores, grocery stores, and pharmacies. Furthermore, for markets without existing retail channels, wholesalers and retailers may be developed and also serve as marketers, educators, and distributors.

Cost Analysis

Currently, it is unrealistic to attempt to determine a final sales price for the possible product of chitosan salts. However, we can give some estimation on both production and distribution cost to see whether the final sales price is acceptable for the end-users. Table 8 demonstrates a comparison between the raw material cost of chitosan acetate (30 mg/L is chosen as dosage for highest average virus removal) and P&G Purifier of Water.

Table 9: Raw Material Cost of Chitosan Acetate and P&G Purifier of Water

	Chitosan Acetate	P&G Purifier of Water
Cost/unit weight ($/kg)	35	8.75
Dosage/unit water (g/10L)	0.3	4(1 sachet)
Cost/unit water (cents/10L)	1.05	3.5

Typically, a four-gram packet of P&G Purifier of Water costs 3.5 cents to make, 4 cents to distribute, and sells for an average of 10 cents at retail. It is notable that the distribution cost accounts for 40% of the sales price of P&G Purifier of Water. For a newly started business, the distribution cost will be much higher to establish a new sales channel. It may be difficult to compete with the established P&G network and achieve a lower distribution cost even through collaborating with PfP and their international distribution network. An important point is that the dosage per unit water of chitosan is much lower than that of P&G Purifier of Water, which may well reduce the transport cost but cannot assure a lower distribution cost.

Marketing Strategies

As a new application, education to encourage behavior change and product use is necessary. To address product use, properly designed instructions are required, which include but not be limited to pictographic material for filter manufacturers and end users in multiple languages based on each target market. Websites will be created with demonstration videos as well as other necessary information. To encourage behavior change, one feasible way is to give out free samples to the first-time users and convert them into regular customers. Other marketing campaigns can be launched based on different markets. Obviously, any kind of promotion campaign requires financing. Therefore, once the prototype is developed, the next step is to seek venture capitalists for potential investment. Finally, local government and NGOs should be involved to facilitate funding subsidies or promote product use.

CHAPTER 7: CONCLUSION

Access to safe drinking water is still unsatisfactory in developing countries, especially in rural areas. Ceramic filter technology for household water treatment is a promising approach to household water quality improvement due to its low-cost, long-term use, and ease of operation to produce a sufficient quantity of water. However, poor virus removal is one of the main concerns of ceramic filters and introducing coagulation as pretreatment can be a solution. After a brief overview of commonly used chemical coagulants, three chitosan salts: chitosan hydrochloride, chitosan lactate and chitosan acetate, were identified as technical options. Experiments were carried out to evaluate the performance of three chitosan salts and results demonstrated that all chitosan salts enhance the performance of ceramic filters, especially on virus removal, with chitosan lactate and chitosan acetate as most promising. Next steps were proposed for further exploration in both laboratory tests and field studies and a brief market plan was developed to describe how this research can be practically applied as a solution to the critical problem of safe drinking water supply.

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